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Study of volatile compounds and antimicrobial activity of Thuja essential oils
Studiul compușilor volatili și activitatea antimicrobiană a uleiurilor esențiale de Thuia

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Abstract

For over 5,000 years, various crops have used these herbal oils for a variety of health problems. The history of the use of essential oils has its roots in antiquity in countries such as Persia, India, Egypt, China. Essential oils are generally formed during the flowering period of plants and accumulate in flowers, fruits, leaves, roots or bark. The chemical composition of an essential oil obtained from a plant differs depending on the parts of the plant from which it was extracted. This paper lists information about Thuja shrubs which includes 5 species of trees, two of which are native to North America (Thuja occidentalis, Thuja plicata) and three from Western Asia (Thuja koraiensis, Thuja standishii, Thuja sutchuenensis). It presents taxonomy, chemical composition, pharmacological properties and notions about Thuja essential oils. The research part presents the identification of volatile compounds in thuja essential oil by GC-MS method and testing the antimicrobial effect of thuja essential oil.

Rezumat

Timp de peste 5.000 de ani, diferite culturi au folosit aceste uleiuri din plante pentru o multitudine de probleme de sănătate. Istoria folosirii uleiurilor esenţiale are rădăcini în antichitate în ţări precum Persia, India, Egipt, China. Uleiurile esenţiale se formează în general în perioada de înflorire a plantelor şi se acumulează în flori, fructe, frunze, rădăcini sau scoarţă. Compoziţia chimică a unui ulei esenţial obţinut dintr-o plantă diferă în funcţie de părţile plantei din care a fost extras. În prezenta lucrare sunt enumerate informații despre arbuștii Thuia unde sunt încadrate 5 specii de arbori, dintre care doi sunt originari din America de Nord (*Thuja occidentalis, Thuja plicata*) și trei din Asia de Vest (*Thuja koraiensis, Thuja standishii, Thuja sutchuenensis*). Este prezentată taxonomia, compoziția chimică, proprietățile farmacologice precum și noţiuni despre uleiurile esențiale de Thuia. În partea de cercetări este prezentată identificarea compuşilor volatili din uleiul esenţial de Thuia prin metoda GC-MS și testarea efectului antimicrobian al uleiului esenţial de Thuia.

Introduction

Essential oils contain a very complex mixture of volatile molecules that can be classified into two main groups, depending on their molecular structure:

1. Terpenes and terpenoids

Terpenes and terpenoids are hydrocarbons formed by combining several isoprene units. While most terpenes do not possess significant antimicrobial activity, many terpenoids are potent antimicrobials.

2. Phenylpropanoid

Phenylpropanoids are a diverse family of molecules that are synthesized from the amino acid’s phenylalanine and tyrosine.

Their antimicrobial activity varies from compound to compound.

There are several mechanisms by which essential oils act on bacteria:

1. Toxicity to the cell wall

Due to the hydrophobic nature of essential oils, they are able to bind to the bacterial wall and act in different ways to disrupt them, increase permeability and cause the loss of cellular components.

Eventually, this leads to the death of bacteria.

2. Action on protein synthesis

The various components of essential oils alter certain protein synthesis pathways.

3. Reduction of intracellular ATP levels

Adenosine triphosphate (ATP) is the "molecular exchange unit" of energy transfer in cells and is vital for cellular metabolism.

4. Reduction of intracellular pH

5. Changes in the cytoplasm

Some essential oils (cinnamon, oregano) cause the protein material to coagulate in the cytoplasm.

Objective:

Considering that the pharmacological and therapeutic properties of an essential oil are given by their chemical components, components that differ depending on the origin, soil, temperature, humidity, etc., we set out to determine the volatile content of the oil commercially available from Thuja and test its antimicrobial effect.

1. General notions about shrubs of the genus Thuja

In Europe they are used more for ornamental purposes [1, 6].

The medicinal parts are represented by leaves, cones and stem, from which tinctures, extracts, essential oils are obtained.

The wood is light, soft and aromatic. It can be easily cut and lasts for decades.

The wood is used to obtain chests for clothes, which are repellent against moths.

Wood can also be used in manufacturing fences or stakes [7].

The wood of the *Thuja plicata* species is used for the manufacture of strings used on guitars. In the 19th century, trees of this genus were used in the form of tincture or ointment to treat warts, warts, dermatophytosis and oral candidiasis.

Natives in Canada used *Thuja occidentalis* leaves to make tea that they consumed to prevent scurvy.

It has been shown to contain 50 mg of vitamin C in 100 g of product.

Native Americans grew *Thuja spp* for wood and medicinal purposes.

They used the preparations from Thuja to treat cough, fever, headache, menstrual disorders, but also muscle pain [16].

Over time, the antiviral, antioxidant, antimicrobial and antidiarrheal effects of *Thuja occidentalis* extracts have been demonstrated.

They have been used to treat bronchial mumps, cystitis, psoriasis, uterine carcinomas, amenorrhea and rheumatism.

The essential oil obtained from these trees contains thujone, a substance that has been studied for its antagonistic effect on GABA receptors [17].

Essential oils or tinctures are currently used, both with antibacterial effect.

Thuja extract has been shown to have an antibacterial effect, acting on both Gram-positive and Gram-negative bacteria [5].

It is most often used to treat acute or chronic upper respiratory tract infections, as an adjuvant antibiotic in severe bacterial infections, such as:

- bronchitis,
- angina,
- pharyngitis,
- otitis media and
- sinusitis.

Immunostimulatory and antiviral properties are based on increased T lymphocyte proliferation and interleukin-2 production.

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1.1. Taxonomic classification of shrubs of the genus Thuja

Thuja spp is a hermaphrodite conifer, classified in:

Genus *Thuja*,
Subfamily *Thujoideae*,
The family *Cupressaceae*, which is part of
Order of *Pinales*,
Class *Pinopsida*,
Division *Pinophyta* [20].

The popular name for this tree is the *"tree of life".* The genus Thuja includes two species native to North America:

- *Thuja occidentalis,*
- *Thuja plicata* and

three species native to Asia:

- *Thuja koraiensis,*
- *Thuja standishii,*
- *Thuja sutchuenensis* [7].

Figure 1. *Thuja occidentalis*
Source: https://www.gardenia.net/plant/thuja-occidentalis-smaragd [35]

Figure 2. *Thuja plicata*
*Source:*https://commons.wikimedia.org/wiki/File:Thuja_plicata_%27Zebrina%27_leaves_01_by_Line1.jpg [33]

Figure 3*. Thuja koraiensis*
*Source:*https://treesandshrubsonline.org/articles/thuja/thuja-koraiensis/ [37]

Figure 4*. Thuja standishii*
Source: https://conifersociety.org/conifers/thuja-standishii/ [34]

Figure 5*. Thuja sutchuenensis*
*Source:*http://www.pinetum.org/sp/THsutchuenensis.html [36]

In Europe the most widespread species is Thuja occidentalis, which is used for ornamental purposes.

This species is found under four major varieties:

- *T. occidentalis var. aureospica,*
- *T. occidentalis var. lutea,*
- *T. occidentalis var. vervaeneana,*
- *T occidentalis var. wareana* [12].

1.1.1. Description of shrubs

Thuja spp is a hermaphroditic conifer, native to North America, a region where it forms vast forests if the ground is wet. It adapts to various climatic and temperature conditions and is resistant to frost [4].

Thuja occidentalis, popularly called "ordinary Thuja" has a straight stem, with short, thick branches with a pyramidal crown.

The bark is thin, smooth, and reddish-brown in color and exfoliates into strips. The vines are flattened and branched alternately in horizontal or oblique planes [25].

The leaves are green, scaly, opposite and completely cover the vine, and the lateral leaves are boat-shaped, and those on the upper and lower face have a resiniferous gland, a gland that gives a characteristic, intense smell and a strong, camphoric taste. .

The leaves on the upper and lower face do not have a convex resinous gland, but a dimple that looks like a scratch. In winter, the foliage turns rusty.

The flowers are unisexual monoecious, arranged in ovoid-elongated cones, 1 cm long, yellow-green and have 3-5 pairs of dry, scaly skin scales, those located at the base are long, almost as long as the cone and mucronate at the tip.

The seeds are small, flat, yellow-brown and have 2 marginal wings, arranged two under each scale. The fruits look like chestnuts.

Thuja orientalis (popular name: "tree of life") is native to the Far East.

The species has a wide ecological distribution, is resistant to drought and smoke, but is not very resistant to frost.

T. orientalis differs from *T. occidentalis* through the stem that branches irregularly from the base and the branches are ascending.

Another difference is the vines, which are thinner and less flattened, arranged in vertical planes.

The cones are larger, 10-25 mm, fleshy, green at first, then brown, frosted, with 6 scales with the tip turned like a horn, which at maturity unfold more laterally. The seeds are brown and wingless [14].

Thuja plicata (popular name: Giant thuja) is native to North America and reaches Alaska. It is an exotic species that can reach up to 60 m in height.

The stem is straight, cylindrical, conical crown, sharp, in isolated specimens being developed to the ground.

The bark is smooth, reddish-brown in color, with an early, exfoliating rhytidome. The main vines are not flattened as in T. occidentalis, but are almost round, the terminal ones flattened, with dense and dense branches.

The leaves are scaly, opposite, green and over the winter, without convex resinous glands, upside down with 2 stripes of bluish stomata.

1.2. Chemical composition

Chemically, the tree contains a number of compounds: saponins, phenols, tannins, amines, mucilage, bitter principles, lactone components, carotenes, essential oils, triterpenes, steroids, reducing carbohydrates, coumarins, tannic acid, polysaccharides, proteins and minerals.

Of all these components, flavonoids and lignans are representative of this group of trees.

From the group of flavonoids have been described:

- catechin,
- gallo catechin,
- mearusitrine,
- myricetin,
- procyanidin B-3,
- prodelphinidin,

- quercetin,
- quercitrin and
- camphor [2].

Among the bioflavonoids were highlighted: bilobetin and amentoflavone.

Amentoflavone is present in a considerable amount in *T. occidentalis* and has high antifungal activity against fungi with high pathogenicity and antiviral properties against respiratory syncytial virus.

It belongs to the group of *carbohydrates*:

- cellulosic polysaccharides and
- non - cellulose (e.g., xylan and mannose).

Water-soluble carbohydrates are represented by:

- arabino galactose and
- pectin.

Lignans are polyphenols and over time have been associated with the antioxidant, antiviral, antibacterial, insecticidal, nematocidal activity of *Thuja spp.*

Isolated lignans from *T. occidentalis* are represented by:

- matairesinol,
- thuja plicata metal ether,
- wikstrom,
- 8-hydroxy thuja plicata metal ether,
- 4-O-demethylatein,
- epi-pinoresinol,
- pinoresinol and
- secoisolariciresinol,

all these being isolated from the xylem of the branches [22].

Thujalignans and lignans are representative metabolic products of the hydrophilic extract obtained from T. occidentalis noted for their antioxidant activity. [2].

1.3. Pharmacological properties

Thuja occidentalis has various pharmacological properties. They are exerted by active substances such as:

- α-pinene,
- d-α-thujone,
- 1-frencona,
- 1-acetic borneol,
- Andisovaleric acid,
- terpineol,
- sabinenes,
- camphor,
- valerian camphoric acid,
- occidol-β-sitoxerol,
- quercetin rhodoxanthin,
- tannins,
- mucilage and
- C vitamin [3, 11].

Thuja spp is thought to have antiviral and immunostimulatory activity.

Thus, it has been shown that the polysaccharides in the composition of preparations obtained from *T. occidentalis* inhibit the activity of the virus:

- human immunodeficiency and a
- influenza A virus.

They act by inducing T lymphocytes, especially CD4 + T lymphocytes [10].

Also, the hepato-protective activity has been demonstrated by the protection it provides to the liver in acute or chronic liver failure, through:

- flavonoids,
- tannins and
- polysaccharides [9].

Phenolic compounds act protectively on the gastric mucosa in lesions caused by aspirin, stress, alcohol or hydrochloric acid [10].

The antidiabetic properties were demonstrated by administering albino Wistar mice a dose of 200mg/kgc., *T. occidentalis* extract which led to a significant increase in glutathione, which has an antioxidant effect.

Improvements were also observed in the body weight and lipid profile of the mice.

It was found that this action is due to the presence of flavonoids, tannic acid, polysaccharides and proteins in this fraction [8].

In an *in vitro* study, the antifungal activity of the homeopathic dilution obtained from *T. occidentalis* against some fungal species was demonstrated*: Aspergillus flavus* and *Aspergillus niger*. It acted on the fungi by inhibiting sporulation and exudation.

In another *in vitro* study, the strong antimicrobial activity of the methanolic extract of *T. occidentalis* against Gram-negative and Gram-positive bacteria (e.g., *Escherichia coli, Citrobacter, Shigella flexenari, Yersinia aldovae, Staphylococcus aureus, Pseudomonas aeruginosa*) and fungi (*Saccharomyces ceréviciae, Aspergillus parasiticus, Trichophyton rubrum, Yersinia aldovae and Candida albicans*) [19, 29].

Constituents of *T. occidentalis* essential oil such as:

- sabinyl acetate,
- fenchone,
- sabien,
- β-thujone,
- α-pinene and
- terpinen-4-ol

have been shown to have antimicrobial activity against *S. aureus, E. coli, E faecalis*, and α-thujone and β-thujone are very active against Gram negative bacteria (*Pseudomonas aeruginosa and Klebsiella pneumonie*).

In studies to determine the inhibitory effect of *T. occidentalis* on *Pseudomonas aeruginosa* using the optical density technique.

It was shown that aqueous and alcoholic extracts of *T. occidentalis* were effective against *P. aeruginosa* at a concentration of 50%, while at a concentration of 10% of these extracts were less effective.

The antibacterial and antimicrobial activity of essential oils obtained from *T. occidentalis* leaves and cones was tested on seven microorganisms:

- *Staphylococcus aureus,*
- *Aeromonas hydrophila,*
- *Escherichia coli,*
- *Pseudomonas aeruginosa,*
- *Salmonella thyphimurium,*
- *Aspergillus flavus and*
- *Aspergillus niger*.

Both oils were effective, but increased microbial activity was observed in the essential oil obtained from the leaves.

The antifungal effect was the strongest. This property of oils is considered to be due to pinene, which is the major component of both oils [15, 23, 25].

1.4. Notions about Thuja essential oils

Essential oils are defined as fragrant liquids extracted from different parts of a plant: flowers, leaves, seeds, stem, and from a chemical point of view, essential oils contain many organic compounds and functional groups.

T. occidentalis essential oil is obtained from leaves or cones and is used in the pharmaceutical industry and in perfumes.

It is obtained by hydro distillation and is used in pharmaceuticals due to its pharmaceutical properties.

It is also used in cosmetics, soaps, deodorants, room sprays, detergents, disinfectants and insecticides [14].

Due to the presence of thujone, which is toxic and may cause dizziness and seizures, the dosage of the oil must be carefully monitored.

Pure thujone is used as an active ingredient in nasal decongestants and antitussives, and due to its anticancer properties can be applied in chemotherapy [27].

There are 3 methods of obtaining essential oils:

1. *Steam distillation* - the usual and most commonly used method.
2. *Solvent extraction*– method to be applied when a heat-sensitive component or containing a major non-volatile constituent is desired.
3. *Extraction from flowers*- method used for delicate flower petals.

In general, the major constituents of essential oils obtained from the leaves of *Thuja spp*., are:

- thujone,
- α-pinene,
- D-3-carene,
- sabinene and
- cedrol [31].

The major constituent of the essential oil is monoterpene thujone, a component with

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pharmacological activity, a component of nasal decongestants, antitussives, perfumes.

The essential oil obtained from *T. plicata* was first studied by Brandel, who reported the presence of the following components: thujone, fenchone and borneol esters.

The presence of the following compounds was observed in the essential oils obtained from *T. occidentalis*:

- borneol,
- camphor,
- limonene,
- myrcene,
- alpha terpinene,
- terpinolene,
- thujyl alcohol,
- carvotanacetone,
- origanol,
- myrcene and camphene,
- sesquiterpene occidenol.

The analysis of the essential oil obtained from *Thuja occidentalis* revealed the presence of 22 compounds, including: α-thujone (49.64%), fenchone (14.06%), β-thujone (8.98%).

Differences were observed in the composition of essential oils obtained from these species.

They can be given by the origin of the trees, the time of harvest, the method of drying, the intensity and duration of mulching, temperature, altitude, season, soil, nutrition and the extraction process.

One study compared the active ingredient content of essential oil obtained from leaves and that obtained from cones.

It has been observed that the essential oil obtained from the leaves has a much more complex structure than that obtained from the cones [23].

The major components of the essential oil obtained from the leaves are: pinene (34.4%), cedrol (13.17%) and phellandrene (8.04%), while in the one obtained from cones predominates pinene (58.55%) and 3-carene (24.08%).

It has been pointed out that the essential oil obtained from the leaves has a very high antioxidant activity [13].

Major differences in the content of active ingredients of essential oils obtained from *T. orientalis* leaves were observed in trees from different regions.

For example, the major components of *T. orientalis* essential oil from Iran are: pinene (21,9%), cedrol (20,3%), D-3-carena (10,5%), and limonene (7,2% while in the Himalayas the major constituents are: pinene (29,2%), D-3-carena (20,1%), cedrol (9,8%), caryophyllene (7,5%) and limonene (5,4,%).

These differences are due to genetic variability, climatic conditions, harvest season, soil composition and drying process [18, 26].

The essential oil obtained from *Thuja occidentalis* in combination with kaolin powder is used for insect fumigation.

Thuja spp. essential oil is part of analgesics, disinfectants, sprays, nasal decongestants.

Due to the characteristic smell that these oils have, they are used in the manufacture of perfumes.

They are also used in the food industry as taste correctors.

Regardless of the industry in which these oils are used, thujone must not be found in the finished product.

For medicinal purposes, the oils are administered externally and should be used with caution, as thujone is neurotoxic.

Symptoms of plug poisoning include: seizures, gastroenteritis, flatulence and hypotension.

The benefits of essential oils are varied.

Thus, applied to the skin they have the effect:

- antirheumatic,
- tonic,
- diuretic and astringent,
- stimulates peripheral nerves and
- reduces irritation.

Inhaled, it acts as a decongestant of the nasal passages, antitussive and expectorant.

Due to the antiviral properties of the components of the oils, it acts as an adjunct in respiratory viruses, reducing their severity and duration.

2. Study of Thuja essential oils

2.1. Materials and methods

2.1.1. Description of thuja essential oil used in the study

The study used essential oil of thuja, a commercially available oil (fig.3.1).

Figure 5. Thuja essential oil

The manufacturer specifies that the essential oils are 100% pure and natural, botanically and biochemically defined, unrectified, undenatured and do not contain synthetic ingredients or solvents.

The oil was obtained by distilling the branches and bark of Thuja with water vapor.

Thuja essential oil has a warm, earthy, earthy aroma.

Provides strong protection against seasonal and environmental harmful factors. It has strong depurative properties and contributes to the proper functioning of cells.

Thuja is also known as the "*Tree of Life*" or "*Arbor vitae*".

It is a powerful cleansing and purifying agent for the body. Thuja essential oil has an anti-inflammatory, antiseptic, antiviral, antidepressant, tonic action.

2.1.2. Identification of volatile compounds in thuja essential oil by the GC-MS method

The analysis of the sample under study was performed using the gas chromatograph model Agilent Technology 7820A (AGILENT Scientific, Santa Clara, CA, USA), coupled with the mass spectrometer MSD 5975 and equipped with a DB WAX capillary column (30 mx 250 pm x 0, 25 pm).

The gas used was helium with a mass flow rate of 1 ml / min.

To separate the compounds, the oven program was used: 40 ° C for 1 min, 5 ° C min-1 to 210 ° C for 5 min.

The injector and ion source temperatures were 250 and 150 °C, respectively.

The injection volume was 1 µl of each pure oil or solvent-free mixture with a 1:20 partition ratio.

The NIST spectrum library was used to identify volatile compounds.

The identification was made by comparing the mass spectra with those stored in the libraries of NIST 02, Wiley 275.

The percentage value of the individual components was calculated based on the peak areas of the GC without using correction factors.

Figure 6. Gas chromatograph Agilent Technology, model 7820A (AGILENT Scientific, CA, SUA)

2.1.3. Testing the antimicrobial effect of Thuja essential oil

Testing of the antimicrobial effect of Thuja essential oil was performed by the method of discs, according to the Standard Standards for Testing the Antimicrobial Sensitivity of Impregnated Discs.

Catalog strains from two Gram-negative bacterial species were used to test the antimicrobial effect:

- *Proteus vulgaris* and
- *Salmonella typhimurium*)

and two Gram-positive species:

- *Staphylococcus aureus* and
- *Bacillus subtilis*.

From the tested bacterial species, young cultures of 24 hours were initially prepared as follows: on a Petri dish with nutritious agar, inoculations were made with the bacteriological loop to obtain isolated colonies.

After incubation in a thermostat for 24 hours at 37 °C from each culture corresponding to the bacterial species used, a colony with a bacteriological loop was taken and passed into 10 ml of nutritious broth.

The broth tubes were then incubated on a thermostat for 24 hours at 37 °C. These were considered fresh and pure crops.

Dilutions were then made from each bacterial species chosen for testing to obtain a cell density of 107 / ml. The density check was performed with the McFarland scale. In order to test the culture medium, the nutrient agar was poured into sterile Petri dishes. One plate was used for each bacterial culture.

After solidification of the medium, the plates were kept at the thermostat for about 15 minutes to remove the condensation that forms on the plate cover due to the temperature difference between the plate and the culture medium.

Then, 1 ml of each prepared culture was seeded on the surface of the culture medium distributed in plates.

By rotating movements, the uniform distribution of the culture on the surface of the environment was achieved. Excess fluid was aspirated.

The plates were then left to stand for 15 minutes for the bacterial bodies to make contact with the culture medium.

Sterile Whatman discs (6mm) were used which were loaded with 20 μl of Thuja essential oil and deposited on the inoculated culture medium.

The plates were incubated at 37 ° C for 24 h. The antimicrobial activity of Thuja essential oil was studied in three concentrations: 25%, 50%, 75% essential oil.

The plates were incubated for 24 hours at 37°C, after which the diameter of the inhibition zone was measured and expressed in mm.

Negative control was prepared using DMSO as solvent, and amoxicillin as positive control. All determinations were performed in triplicate and for statistical analysis the software was used to evaluate a unilateral analysis of variance (ANOVA) at $p \leq 0.05$.

2.2. Results and discussion

2.2.1. Identification of volatile compounds in thuja essential oil by the method GC-MS

Following the gas chromatographic analysis coupled with mass spectrometry, for Thuja essential oil the following compounds were identified in the chromatogram (Fig. 7.), Table 1 and Figure 8.

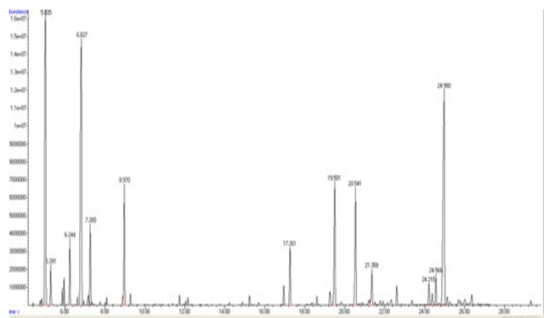

Figure 7 Chromatogram of Thuja essential oil

Table 1.
Volatile compounds identified in Thuja essential oil

Compound	R. I	Conc.%
Monoterpenic hydrocarbons		
1 α -Pinene	935	22,25
Camphene	947	1,32
Sabinene	970	0,54
β -Pinene	974	0,88
β -Myrcene	992	2,16
3-Carene	1012	20,47
p-Cymene	1020	0,49
Limonene	1024	3,38
Terpinolene	1092	4,58
Oxygenated monoterpenes		
Terpinen-4-ol	1172	0,58
α-Terpinol acetate	1280	2,72
Sesquiterpene hydrocarbons		
β -Elemen	1386	0,36
α-Cedrene	1412	0,81
β -Caryophyllene	1416	6,12
α-Humulene	1452	5,57
γ -Cadinene	1476	0,40
δ -Cadinene	1479	0,81
Sesquiterpene oxygenates		
Spathulenol	1511	1,12
α-Cadinol	1541	0,67
Caryophyllene oxide	1581	0,62
Cedrol	1654	19,21

Following the gas chromatographic analysis in Thuja essential oil, 21 components were identified, amounting to 95.06% of the total composition of essential oil and representing four different groups of hydrocarbons, namely: *monoterpenes, hydrocarbons, oxygenated monoterpenes, sesquiterpene, hydrocarbons and oxygenated sesquiterpenes.*

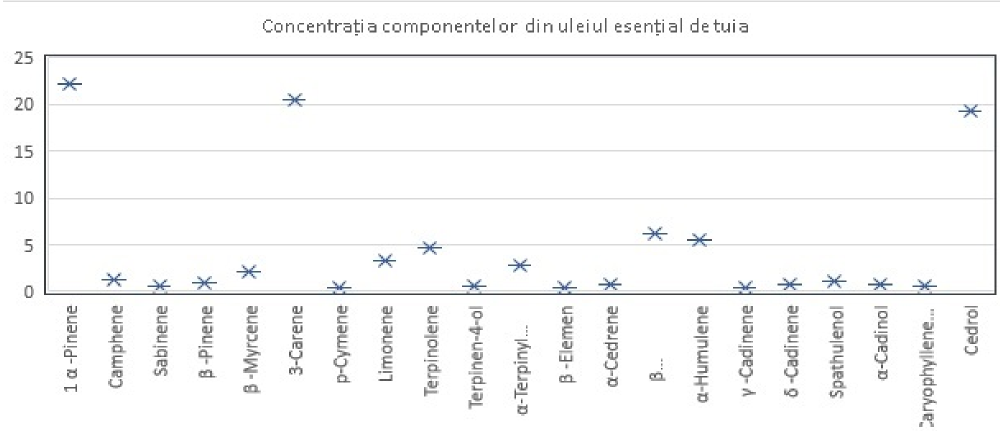

Figure 8. Percentage graphical representation of components in Thuja essential oil

Monoterpenic hydrocarbons were the most dominant chemical group (56.07%) and among the components:

- α–pinene (22,25%), followed by
- 3-carene (20,47%),
- terpinolene (4,58%),
- limonene (3,38%),
- β-myrcene (2,16%) and
- camphene (1,32%).

Oxygenated monoterpenes account for 3.3% of the components of Thuja essential oil, and the highest proportion was α-terpinyl acetate (2.72%).

Sesquiterpene hydrocarbons were 14.07%, including as predominant compounds:

- B-caryophyllene (6,12%) and
- α-humulene (5,57%).

Oxygenated sesquiterpenes represent 21.62% of the composition of Thuja essential oil and had as major components:

- cedrol (19,21) and
- spathulenol (1,12).

The composition of the essential oil showed some similarities with previous studies conducted by other researchers, but with differences in the region in which the plant was grown.

For example, the major components of *T. orientalis* essential oil from Iran are:

- pinene (21,9%),
- cedrol (20,3%),
- D-3-carene (10,5%), and
- limonene (7,2%),

while in the Himalayan the major constituents are:

- pinene (29,2%),
- D-3-carene (20,1%),
- cedrol (9,8%),
- caryophyllene (7,5%) and
- limonene (5,4,%).

These differences are due to genetic variability, climatic conditions, harvest season, soil composition and drying process [32].

Another study conducted in Pakistan showed that Thuja essential oil has 22 main components represented by:

- α-pinene (40,6 %),
- beta-caryophyllene (6.8%),
- cedrol (10.7 %),
- alloaromadendren (7.8%),
- β -myrcene (3,7 %) and
- R-+-limonene (3.2%),

and another study conducted in Egypt highlights 23 main components, the most predominant being:

- α pinene (21,83%),
- β-pinene (6,71%),
- β-caryophyllene (12,07),
- α-cedrol (6,86%),
- β-selinene (6,15%), and
- limonene (5,49%) [18, 28].

2.2.2. Testing the antimicrobial effect of Thuja essential oil

The *in vitro* antimicrobial activity of Thuja essential oil against microorganisms was evaluated qualitatively and quantitatively by the microdilution method.

The zones of inhibition against the microorganisms tested are shown in table 2 and fig. 9.

Table 2.
The results of the antimicrobial effect for Thuja essential oils

	Inhibition zone halo diameter (mm)			
	Gram positives			
Concentration	**25%**	**50%**	**75%**	**Amoxicillin**
Bacillus subtilis	12,6±0,14	14,8±0,11	30,12±0,16	39,4±0,11
Staphylococcus aureus	21,8±0,12	24,18±0,12	35,42±1,08	NA
	Gram negatives			
Proteus vulgaris	NA	NA	18,05±0,7	42,2±0,32
Salmonella typhimurium	NA	NA	NA	15,4±0,18

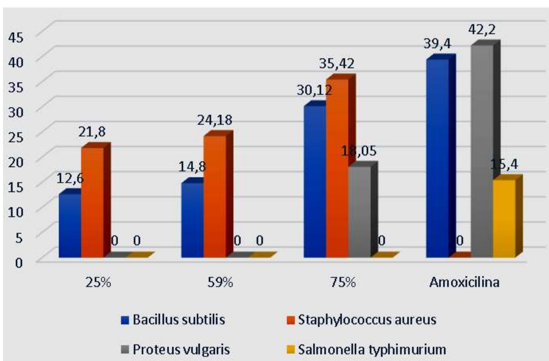

Figure 9. Graphical representation of the inhibition zone (mm) at the three concentrations of Thuja essential oil

The results obtained for Thuja essential oil showed that it has the highest activity against gram-positives, the genera:

- *Staphylococcus aureus* (35,42 ±1,08) and
- *Bacillus subtitilis* (30,12 ±0,16),

while, it showed no activity against *Salmonella typhimurium* (gram negative), and in the case of the gram-negative pathogen *Proteus vulgaris*, the antimicrobial activity was obtained only at the maximum applied concentration (18.05 ±0.7 compared to 42.2 ±0,32 to amoxicillin).

The results obtained in this study are similar to those of other researchers who support the antimicrobial activity of Thuja essential oil, especially against gram-positive pathogens. [24, 31].

2.3. Conclusions

Following the study on Thuja essential oil on chromatographic analysis and testing of antimicrobial efficacy, we can conclude that:

- Thuja essential oil has significant inhibitory effects against gram-positive and gram-negative bacteria, which are associated with clinical diseases;
- it is more effective against gram positives than gram negatives;
- inefficient against *Salmonella typhimurium;*
- the major components identified were:
  - α–pinene,
  - 3-carene,
  - terpinolene,
  - limonene,
  - β-myrcene,
  - camphene and,
- the presence of these compounds shows that Thuja essential oil has great potential for use in many medical applications.

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